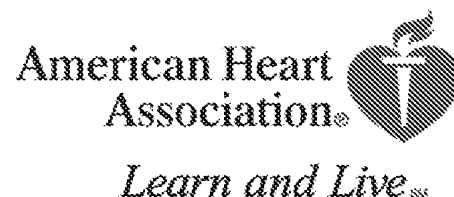


EXHIBIT A

Circulation

JOURNAL OF THE AMERICAN HEART ASSOCIATION



Different Effects of Angiotensin Receptor Blockade on End-Organ Damage in Salt-Dependent and Salt-Independent Hypertension

Karlene Maitland, LaKeesha Bridges, Wendell P. Davis, Joseph Loscalzo and Mildred A. Pointer

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Different Effects of Angiotensin Receptor Blockade on End-Organ Damage in Salt-Dependent and Salt-Independent Hypertension

Karlene Maitland, BS; LaKeesha Bridges, BS; Wendell P. Davis, DVM;
Joseph Loscalzo, MD, PhD; Mildred A. Pointer, PhD*

Background—Although angiotensin II type 1 receptor blockers have emerged as effective antihypertensive agents, it is not known how efficacious these agents are in treating hypertension-associated target organ damage.

Methods and Results—The present study was undertaken to compare the effect of angiotensin type 1 receptor inhibition on the progression of the organ damage observed in 2 models of hypertension, namely, salt-sensitive and nitric oxide synthase inhibition-mediated hypertension. Effective (16.4 $\mu\text{mol/kg}$) and ineffective (0.8 to 4.9 $\mu\text{mol/kg}$) antihypertensive doses of candesartan cilexetil were initiated after hypertension was established. Both low- and high-dose candesartan cilexetil significantly reduced cardiac and renal damage in the nitric oxide synthase inhibitor model of hypertension ($P<0.05$ versus untreated); however, high-dose candesartan caused a significant increase in renal damage in the Dahl salt-sensitive model of hypertension ($P<0.05$ versus untreated). Interestingly, the beneficial end-organ effects of candesartan in the nitric oxide synthase inhibition model were independent of sustained antihypertensive actions of candesartan, whereas the exacerbation of renal injury with candesartan in the Dahl salt-sensitive model was inversely related to its blood pressure-lowering effect.

Conclusions—These data show that angiotensin type 1 blockade reduces injury in the L-nitroarginine methyl ester model but increases tissue injury in the salt-sensitive model. These data suggest that angiotensin II via angiotensin type 1 receptor activation contributes to organ damage in nitric oxide-deficient salt-independent hypertension but is protective in salt-induced hypertension. These data further suggest that (1) renal injury may evolve independently of blood pressure and (2) the effectiveness of an antihypertensive agent in ameliorating renal injury may depend on the etiology of the hypertension. (*Circulation*. 2006;114:905-911.)

Key Words: angiotensin ■ angiotensin II type 1 receptor blockers ■ end-organ damage ■ hypertension ■ nitric oxide ■ sodium chloride, dietary

Despite reductions in end-organ complications among hypertensive subjects from 1970 to 1990, recent evidence indicates that the incidence of coronary heart disease, heart failure, and stroke has been rising in the last 10 years.¹ Interestingly, end-stage renal disease has more than doubled in the past 20 years.^{1,2} The reason for the rise in end-organ damage associated with hypertension is not clear. It has been suggested that part of the reason for the steady rise in incidence of cardiovascular disease may be due to a decrease in patient compliance and/or awareness of the complications of hypertension, failure of physicians to provide treatment plans that would achieve blood pressure normalization, or both.^{1,3} Alternatively, the recent trend in cardiovascular disease rates may suggest that the mechanism(s) involved in end-organ damage may be different from those involved in hypertension per se, and the earlier

decline in cardiovascular deaths is the result of better health practices in general and improved medical and technological interventions for the treatment of end-organ complications.

Clinical Perspective p 911

Compared with other populations, blacks have a 3- to 17-fold greater incidence of end-stage renal disease.^{4,5} This finding coupled with the fact that $\approx 75\%$ of black hypertensives are salt-sensitive suggests that salt may be a critical determinant of the development of renal damage associated with hypertension.⁶ Angiotensin II type 1 receptor blockers have recently emerged as effective antihypertensive agents⁷; however, the efficacy of these agents in preventing long-term, hypertension-associated, end-stage organ disease is not known. The present studies were designed to investigate (1) the role of angiotensin II (Ang II) in

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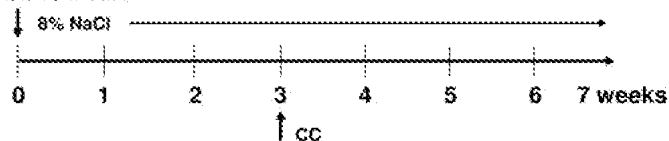
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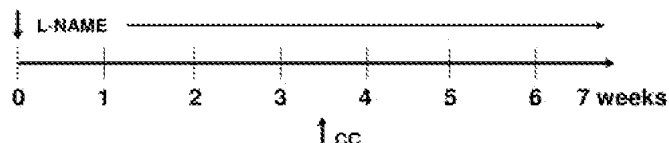
EXPERIMENTAL PROTOCOL

Series I (0.5–1 mg/kg CC)

Dahl Salt-sensitive Model



L-NAME Model



Series II (10 mg/kg CC)

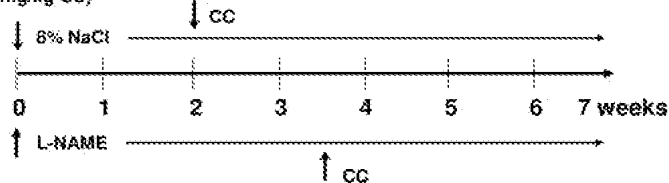


Figure 1. Experimental protocol for salt-dependent (DS) and salt-independent (DR plus L-NAME) models of hypertension in series I and series II.

the tissue damage that usually accompanies chronic hypertension and (2) the effect of angiotensin type 1 (AT_1) receptor blockade on tissue damage as a function of salt-sensitive and non-salt-sensitive hypertension. In this study we compared the effects of Ang II type 1 receptor blockade in 2 models of hypertension: salt-dependent and nitric oxide synthase inhibition-dependent. We used the Dahl salt-sensitive (DS) rat on a high-salt diet as a salt-dependent model and the Dahl salt-resistant (DR) rat on a low-salt diet treated with L-nitroarginine methyl ester (L-NAME) as a model of hypertension dependent on nitric oxide synthase inhibition.

Methods

Animals

All animal study protocols were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Male DS and DR rats (inbred/Rapp, 5 to 6 weeks of age) were purchased from Harlan Sprague-Dawley (Harlan, Indianapolis, Ind). All animals were maintained on a low-salt (0.12% NaCl) diet as administered by the vendors and allowed a 3- to 5-day acclimation period before basal blood pressures were measured.

Experimental Protocol

Series I

After basal blood pressures were measured, rats were assigned to 1 of 3 treatment groups. The total duration of the study was 7 weeks: 3 weeks of hypertension followed by 4 weeks of treatment. Weekly tail-cuff blood pressure measurements were made throughout the study period.

Salt-Sensitive Model

DS rats received either 8% NaCl diet alone ($n=12$), 8% NaCl plus 0.8 to 4.9 $\mu\text{mol/kg}$ per day (0.5 to 3 mg/kg) candesartan cilexetil (CC) ($n=15$), or a low-salt (0.12% NaCl) diet ($n=7$). In those animals receiving CC, the protocol consisted of 3 weeks on a high-salt diet alone before CC was initiated (0.8 to 4.9 $\mu\text{mol/kg}$ per day in drinking water) followed by 4 weeks of CC treatment (Figure 1).

L-NAME Model

DR rats maintained on a low-salt (0.12% NaCl) diet were made hypertensive with the use of L-NAME (148 $\mu\text{mol/kg}$ per day)

administered in drinking water. Once blood pressure had increased to a level comparable to that of salt-sensitive rats (between weeks 3 and 4 of L-NAME treatment), CC was initiated and continued for 3 to 4 weeks (Figure 1). There were 3 treatment groups: L-NAME alone ($n=12$), L-NAME plus 0.8 to 4.9 $\mu\text{mol/kg}$ per day CC ($n=8$), and untreated low salt ($n=6$).

Series II

A second series of animals was studied to address 2 concerns raised by series I, namely, the difference in the severity of organ damage between the 2 models and the difference in the time period before euthanasia between the group treated with high salt alone and the group treated with high salt plus CC. Thus, in an effort to minimize the difference in the degree of organ damage between the 2 models and to eliminate the disparity in study duration between untreated and CC-treated DS rats, we initiated CC treatment after only 2 weeks of 8% NaCl compared with 3 weeks in series I. Series I data suggested that the organ damage in DS rats might be due to the sustained elevated blood pressure, unlike the L-NAME model. Therefore, an effective blood pressure-lowering dose of CC (16.4 $\mu\text{mol/kg}$ per day, which is equivalent to 10 mg/kg per day) was given to both hypertensive models.

Blood Pressure Measurement

Weekly tail-cuff systolic blood pressures were measured with the use of an IITC (model 20NW; Woodlands, Calif) or Visitech (model BP2000; Apex, NC) monitoring system.

Histology

At the end of the study, animals were anesthetized and perfused with phosphate-buffered saline and subsequently with 10% formalin through a left ventricular puncture site. Hearts and kidneys were removed and weighed before placement in sample vials containing 10% formalin. All histological slide preparation and staining were performed by Histotechniques (Powell, Ohio) with the use of conventional fixative, sectioning, and staining procedures. Kidney slices were stained with PAS, and hearts sections were stained with Masson trichrome stain.

Pathology Scoring

All pathology evaluations were performed by a pathologist blinded to treatment. Each tissue slice was scored for grade of tissue injury (0 to 4, with 0 representing no abnormality and 4 representing maximal pathological changes), as well as extent of the tissue injury (1 to 3, with 1 representing focal, 2 indicating multifocal, and 3

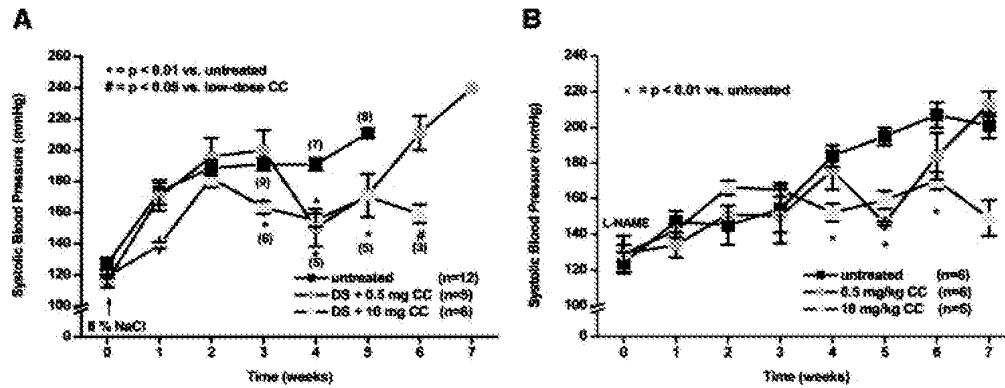


Figure 2. Effect of CC on the time course of blood pressure response in the 2 models of hypertension. High salt (8% NaCl) or L-NAME administration was begun at week 0. CC was initiated after 3 weeks of high salt in the low-dose (0.5 to 3 mg/kg) group and at week 2 in the high-dose (10 mg/kg) group (left). CC was initiated in the L-NAME rats once a blood pressure comparable to that of the salt-sensitive hypertension model was achieved (between 3 to 4 weeks after L-NAME) (right).

representing diffuse or global pathological changes). Each tissue specimen was examined for glomerular damage (thrombosis, cellularity, fibrosis, sclerosis), tubular damage (protein casts), interstitial inflammation (leukocyte infiltration), and vascular changes (intimal and medial hypertrophy as well as perivascular inflammation). Cardiac injury was assessed as necrosis, fibrosis, vascular injury (intimal proliferation and medial hypertrophy), and perivascular inflammation (cellular infiltration). The pathology score was calculated as the product of injury score (grade) times the extent of injury score (distribution) for each injury type. These products were summed for the final pathology score. Myocyte size was measured with the use of Image Pro Plus 3.0 software (Media Cybernetics, The Imaging Experts; Silver Spring, Md) that calculated area (arbitrary units) from transverse section cell outline tracing. With the use of cells with a clearly definable central nucleus, an average area value was determined from several tracings within each $\times 40$ field.

Morbidity/Mortality

Morbidity in this study was defined as the presence of at least 1 of the following characteristics: apparent paralysis in the front paws leading to an inability to eat, reduced mobility without any apparent paralysis, scruffy coat, and ocular discharge. Mortality was defined as death occurring before the prescribed study protocol. This included spontaneous death as well as death caused by humane euthanasia because of morbidity as defined above.

Statistical Analysis

All data are expressed as mean \pm SE. Comparisons among the treatment groups were analyzed by 1-way or 2-way ANOVA followed by Dunnett multiple comparison tests. Statistical significance was determined at a probability value of 0.05. Only animals

that completed the protocol in the CC groups were considered for analysis in the tissue injury and hypertrophy data analysis.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Blood Pressure

Figure 2 shows the blood pressure response in the salt-dependent and salt-independent models of hypertension after CC treatment. As shown in Figure 2, left, the low-dose CC caused only a transient decrease (-40 ± 10 mm Hg) in pressure after the first week of administration in the DS rats. By the third week of low-dose CC administration, the pressure returned to pretreatment levels and continued to rise during the fourth week of antihypertensive treatment, peaking at 240 ± 5 mm Hg (maximal reading of instruments) ($n=5$); however, the high-dose CC caused a sustained decrease in blood pressure, with a maximal reduction occurring in the second

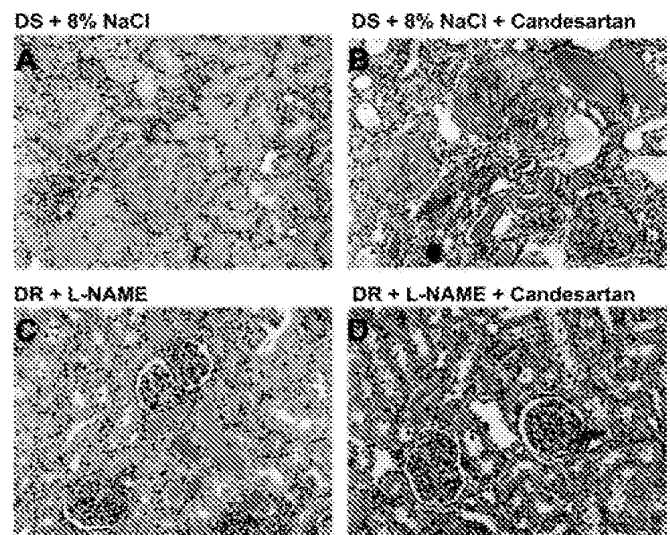


Figure 3. Renal histology in the DS (A and B) and DR L-NAME (C and D) models of hypertension. A and C are without CC, and B and D are with 0.5 to 3 mg/kg per day CC. The salt-sensitive rat received 8% NaCl for 3 weeks before initiation of CC, and the L-NAME (40 mg/kg per day) rat was given L-NAME for 3 to 4 weeks before CC administration.

TABLE 1. Mortality in DS and L-NAME Models of Hypertension

Group	n	No. of Animals	
		Morbidity	Mortality
DS+NaCl	14	5 (36%)	0
+0.5 mg/kg CC	12	7 (58%)	0
+10 mg/kg CC	9	0	0
L-NAME	10	3 (30%)	4 (40%)
+0.5 mg/kg CC	6	0	0
+10 mg/kg CC	5	0	0

Experimental design is described in Methods. Morbidity is defined as loss of mobility, appetite, and weight. Mortality is defined as spontaneous death before protocol point.

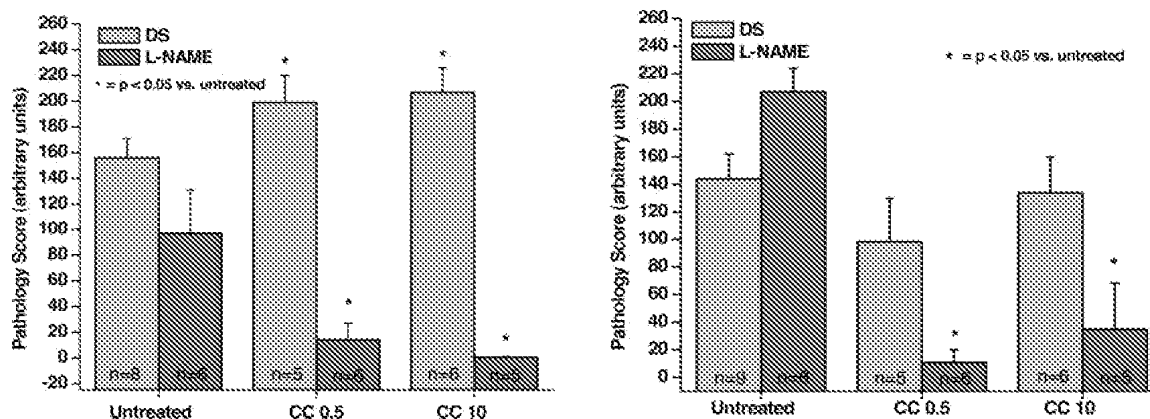


Figure 4. Pathology index scores in renal (left) and cardiac (right) tissues from the DS (light bars) and L-NAME (dark bars) models of hypertension. CC was administered at 0.5 to 3 mg/kg per day or 10 mg/kg per day.

week of treatment (191 ± 3.8 to 155 ± 7.8 mm Hg). Similarly, in the low-salt L-NAME model, the low-dose CC caused a transient reduction of blood pressure, whereas high-dose CC caused a sustained decrease in blood pressure of 41 ± 6 mm Hg over the 4-week period (Figure 2, right). As seen in the salt-sensitive model, there was a significant transient decrease in blood pressure after the first week of low-dose CC in the L-NAME hypertensive model (146 ± 1 versus 195 ± 5 mm Hg in untreated rats at same time period; $P < 0.05$).

Morbidity/Mortality

Interestingly, there was a significant increase in morbidity rate (36% versus 58%; $P < 0.01$) with low-dose CC in the DS animals (Table 1). High-dose CC completely prevented morbidity and mortality normally seen in these animals after 3 to 4 weeks of high salt. In contrast, low-dose CC completely prevented the morbidity and mortality normally seen with L-NAME treatment alone; high-dose CC showed a similar benefit in the L-NAME model.

Tissue Pathology

Glomerular damage, tubular casts, and inflammatory cell infiltration in renal tissue were seen in both models of hypertension (Figure 3A and 3C). After low-dose CC, there was no observed renal damage in the L-NAME model (Figure 3D); however, renal damage persisted in the salt-sensitive model (Figure 3B). Although our protocol was designed to minimize disparate renal injury between

models by controlling the degree and duration of hypertension before initiating treatment with CC, we still observed differences in the degree of renal damage between the 2 models. We reasoned that the difference between models might be accounted for by differences in time of onset of hypertension, aggressiveness of the pathology, and blood pressure–dependent injury effects. Furthermore, the difference in pathology between untreated DS rats and low-dose CC–treated DS rats may be due to the difference in age at the time of euthanasia. Notably, the untreated DS rats on a high-salt diet became ill at week 4 or 5 and were unable to complete the prescribed 7-week protocol. To address these concerns, we performed a second series of experiments that (1) shortened the time to CC initiation in the DS rats in an effort to render the pathology comparable to that of L-NAME hypertensive rats and (2) increased the dose of CC to assess the blood pressure dependency on renal injury in each model.

Figure 4 shows summarized renal and cardiac pathology in the 2 models of hypertension for series I (low-dose CC) and series II (high-dose CC). There was no amelioration of renal damage in the DS model given 10 mg/kg per day ($16.4 \mu\text{mol/kg}$) (Figure 4, left) despite a significant reduction in blood pressure (Figure 2, left). The DS rats on high-salt and high-dose CC were of an age at the time of euthanasia comparable to that of the DS rats in the untreated group since all were the same age when entered into the study and study duration was comparable between the 2 groups (32 ± 2 versus 34 ± 3 days of study duration for untreated and high-dose CC–treated rats,

TABLE 2. Specific Renal Injury in DS and L-NAME Models of Hypertension

Treatment Group	Glomerular Damage	Tubular Damage	Interstitial Inflammation	Perivascular Inflammation	Vascular Injury
HS alone (n=8)	77±5	5±1	13±3	12±3	17±4
HS+low-dose CC (n=5)	82±9	7±1	18±2	18±2	13±3
HS+high-dose CC (n=6)	91±15	6±1	24±2*	24±2*	34±4*
L-NAME alone (n=6)	39±15	3±1	6±2	7±2	13±4
L-NAME+low-dose CC (n=6)	0±0*	0±0*	1±1*	0±0*	5±5
L-NAME+high-dose CC (n=5)	0±0*	0±0*	0±0*	0±0*	0±0*

Values equal mean±SEM. HS indicates high salt. Glomerular damage includes presence of fibrosis, sclerosis, and thrombosis; tubular damage includes protein casts; inflammation includes cellular infiltration; and vascular injury includes medial hypertrophy and intimal proliferation.

* $P < 0.05$ vs high salt or L-NAME alone.

TABLE 3. Kidney or Heart: Body Weight Ratios in DS Rats and L-NAME-Treated Rats

Group	n	Kidney	Heart
		% of Body Wt	% of Body Wt
DS			
Untreated	9	1.23±0.05	0.52±0.02
0.5 mg/kg CC	5	1.44±0.17	0.74±0.08*
10 mg/kg CC	6	1.39±0.12	0.52±0.03†
L-NAME			
Untreated	6	0.97±0.07	0.48±0.04
0.5 mg/kg CC	3	0.89±0.07	0.41±0.01
10 mg/kg CC	3	1.09±0.06	0.38±0.01*

Values are given as mean±SD. Untreated indicates 8% NaCl or L-NAME alone.

* $P<0.05$ vs untreated.

† $P<0.05$ vs CC 0.5 mg/kg group.

respectively; $P=NS$). Therefore, despite comparable age and duration on the high-salt diet (initiated at only 2 weeks of high salt) and a significant reduction in blood pressure, the high-dose CC-treated animals had greater renal damage than untreated hypertensive salt-sensitive rats (Figure 4, left; $P<0.05$ versus untreated). Table 2 shows the specific renal injury observed in each model. Although glomerular damage in the salt-dependent model tended to increase with increasing doses of CC, it was not significant. On the other hand, there was a dose-dependent increase in inflammation and vascular injury in this model. In contrast to the salt-dependent model, both doses of CC effectively ameliorated the glomerular injury, tubular casts, and inflammation in the L-NAME rats. As shown in Table 3, no renal hypertrophy was associated with either model of hypertension under these conditions, and CC had no effect on this response.

Cardiac injury was evident in both models of hypertension (Figure 4, right). As with renal injury, L-NAME hypertensive rats did not show any evidence of cardiac damage (necrosis, fibrosis, and vascular injury) with low-dose CC despite continued hypertension (Figure 4, right). The high-dose CC-treated rats similarly were without any significant cardiac injury compared with untreated L-NAME rats (Figure 4, right; Table 4). Interestingly, although the low dose ameliorated the cardiac injury (Figure 4, right; Table 4) associated with hypertension in the L-NAME model, it did not significantly reduce cardiac

hypertrophy (Table 3). However, the reduced cardiac injury was accompanied by a significant reduction in cardiac hypertrophy with the blood pressure-lowering high-dose CC (Table 4). Similarly, myocyte size (Table 4) was reduced, although not significantly, by both doses of CC in the L-NAME model (9151 ± 1951 versus 5412 ± 622 and 5635 ± 541 for untreated versus low-dose CC and high-dose CC, respectively).

The effect on cardiac injury was quite different in the salt-sensitive model. Specifically, DS animals treated with low-dose CC had increased cardiac hypertrophy, whereas high-dose CC resulted in virtually no change in the cardiac hypertrophy normally observed in the untreated hypertensive rats (Table 3). Interestingly, specific cardiac injury (Table 4) tended to be reduced with the low-dose CC, but this was not statistically significant. The high dose reversed this trend of the low-dose CC (Table 4). Although myocyte size tended to increase with the low-dose CC (8966 ± 1244 versus $10\,796\pm1539$), there was no significant difference among the treatment groups (Table 4).

Discussion

The results of this study reveal that AT₁ receptor activation is likely involved in the end-organ damage associated with L-NAME-induced hypertension but not in salt-induced hypertension. Most interestingly, the beneficial effects of Ang II type 1 receptor inhibition with CC on cardiac and renal pathology in the L-NAME model of hypertension were independent of sustained antihypertensive actions of CC. In contrast, CC did not prevent the end-organ damage seen in the DS model. Indeed, tissue injury continued to progress despite a lowering of blood pressure with the higher dose of CC. These data suggest that (1) the processes involved in end-organ damage associated with hypertension may differ from those leading to the development and maintenance of hypertension per se and (2) the mechanism of tissue injury may differ among models of hypertension.

Despite the significant reduction in cardiovascular morbidity and mortality in the 1970s and 1980s, the mortality rates for stroke, heart failure, and end-stage renal disease associated with hypertension have been on the upswing throughout the past decade.¹ Much of the focus of hypertension management has been on reducing blood pressure in the hope that this will result in improvement in cardiovascular disease. Although considerable evidence indicates a correlation between blood pressure reduction and cardiovascular benefit,^{3,8–10} control of blood pressure has not resulted in the fully predicted decrease in

TABLE 4. Specific Cardiac Injury in Salt-Sensitive and L-NAME Models of Hypertension

Treatment Group	Necrosis	Fibrosis	Vascular Injury	Perivascular Inflammation	Myocyte Area
HS alone (n=8)	1.5±0.8	3.1±0.8	6.5±0.6	4.6±0.8	8966±1244
HS+low-dose CC (n=5)	0.8±0.5	2.0±0.9	4.4±1.7	2.6±1.2	10796±1540
HS+high-dose CC (n=6)	2.7±0.4†	4.4±1.0	7.2±1.1	5.2±1.0†	7076±862
L-NAME alone (n=6)	6.3±1.2	4.3±0.3	7.7±1.1	7.3±1.5	9151±1951
L-NAME+low-dose CC (n=6)	0.3±0.3*	0.7±0.4*	0.7±0.7*	1.3±0.8*	5412±622*
L-NAME+high-dose CC (n=5)	0.8±0.8*	0.8±0.8*	2.4±1.2*	0.8±0.8*	5635±541*

Values are mean±SEM. HS indicates high salt. Vascular injury includes medial hypertrophy and intimal proliferation; perivascular inflammation includes cellular infiltration.

* $P<0.05$ vs L-NAME alone.

† $P<0.05$ vs low-dose CC.

coronary artery disease and end-stage renal disease.^{11,12} Lack of adequate blood pressure control is believed to contribute to these disparate observations.¹³ However, lack of adequate blood pressure control may not explain all of the increase in cardiovascular and renal disease observed over the last 10 years in the hypertensive population.^{14,15} Remarkably, renal disease has more than doubled in incidence between 1982 and 1995,¹ a time during which other cardiovascular disease is declining. Furthermore, blood pressure control in blacks is often not associated with an improvement in renal function.¹⁶

Thus, the mechanism(s) of target-organ damage may involve processes that differ in part from those that cause hypertension. As seen in the L-NAME model of hypertension, subpressor doses of CC eliminated any histological evidence of cardiac or renal damage despite significantly elevated blood pressures.

It is important to note that because the progression of the disease in the salt-sensitive model appeared to be more severe, we shortened the time to initiation of CC from 3 weeks to 2 weeks in series II, thus making the total time of observation comparable between untreated and treated animals (32 ± 2 versus 34 ± 2 days). We previously reported that renal function¹⁷ and structure¹⁸ are not disturbed after 1 week of high-salt treatment. Thus, the pathological changes observed are likely to occur after 1 week of salt administration. Additionally, we reasoned that the pathology in the salt-sensitive model might be associated with the hypertension, whereas that of the L-NAME model is not. Consequently, we used a blood pressure-lowering dose of CC. Despite shortening the time on high salt to 2 weeks and lowering blood pressure, CC did not offer any observable histological benefits. Indeed, DS rats with only 2 weeks of high salt and 3 weeks of CC showed significantly greater tissue damage than DS rats on high salt for 3 weeks plus 4 weeks of low-dose CC, despite a reduction in blood pressure in the former group. Interestingly, there was no evidence of morbidity or death in this group despite the worsening pathology. More investigation is required to determine the exact cause of the morbidity and mortality in this model.

It is not clear how AT₁ receptor blockade leads to an aggravation of the tissue injury observed in the salt-sensitive model of hypertension. However, similar findings of increased tissue injury with angiotensin receptor blockade have been reported in clinical trials.^{19–23} The Candesartan in Heart failure Assessment of Reduction of Mortality and morbidity (CHARM)-Alternative study revealed a 36% increase in myocardial infarction with candesartan versus placebo.²⁰ Greater renal dysfunction was also observed in patients receiving candesartan. More recently, Cheung and colleagues performed a meta-analysis of 3 large clinical trials comparing angiotensin receptor blockers against other antihypertensive agents and found that angiotensin receptor blockers increased myocardial infarction.^{21–23}

The most likely²⁴ candidate to explain these effects is the activation of the Ang II type 2 receptor (AT₂). Much of the current evidence suggests that the AT₂ receptor acts in opposition to the AT₁ receptor. For example, AT₂ receptor activation appears to evoke antiproliferative, angiogenic, vasodilatory, and natriuretic effects,^{25–28} all of which are counter to those of AT₁ stimulation. Interestingly, studies in AT₂ knockout mice suggest a protagonist action of AT₂ receptor activation in aortic banding

hypertension. Specifically, cardiac hypertrophy is prevented in AT₂ receptor knockout mice²⁹ during hypertension due to aortic banding.

Activation of this receptor due to the elevated angiotensin level may account for the responses observed in the L-NAME model of hypertension. Both renal and cardiac injuries are ameliorated with low- and high-dose CC. Low-dose CC reduced cardiac hypertrophy, whereas the high dose completely eliminated cardiac hypertrophy despite elevated blood pressure. Although several studies have shown that AT₂ receptor activation leads to an NO-mediated increase in cGMP,^{30,31} our data suggest that the diminished cardiac hypertrophy occurs independent of NO-cGMP in the L-NAME model. In contrast, the salt-sensitive model may have normal or decreased AT₂ receptor activation due to high salt-induced low circulating Ang II level. We measured plasma Ang II in some of the DS rats on a high-salt diet and in DR rats on L-NAME plus a low-salt diet. As expected, plasma Ang II level was 90 ± 21 pg/mL ($n=4$) in DS rats and 300 ± 100 pg/mL ($n=6$) in DR rats. Consequently, the low Ang II levels may be insufficient to activate the AT₂ receptors, resulting in a counteraction or reversal of the effects of other processes involved in the tissue injury associated with this model of hypertension. It is interesting to note that cardiac hypertrophy was significantly reduced with the higher dose of CC only. Our data do not address whether the beneficial effects on cardiac hypertrophy were due to direct effects of AT₂ activation, indirect effects of reducing blood pressure, or both; however, the higher dose of CC was associated with even greater, although not significant, cardiac tissue damage. Taken together, these observations suggest that there may be differential effects of AT₁ blockade on cardiac hypertrophy and tissue fibrosis. It is possible that some of the tissue injury in this model may be due to direct actions of increased dietary salt.^{32–34} Although not measured in these animals, tissue aldosterone may be involved in the tissue injury. Several studies reveal that aldosterone inhibitors are protective against organ damage in hypertension.²⁴

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Disclosures

None.

References

1. Sheps SG. Overview of JNC VI: new directions in the management of hypertension and cardiovascular risk. *Am J Hypertens*. 1999;12:65S–72S.
2. McClellan W, Tuttle E, Issa A. Racial differences in the incidence of hypertensive end-stage renal disease (ESRD) are not entirely explained by differences in the prevalence of hypertension. *Am J Kidney Dis*. 1988;12:285–290.

3. JNC VI. Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (JNC VI): the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Arch Intern Med.* 1997;157:2415–2446.
4. Shulman N, Ford C, Hall W, Blafox M, Simon D, Langford H, Schneider K. Prognostic value of serum creatinine and effect of treatment of hypertension on renal function: results from the Hypertension Detection and Follow-up Program. *Hypertension.* 1989;13(suppl 1):I80–I93.
5. Rostand S, Kirk K, Rutsky E, Pate B. Racial differences in incidences of treatment for end stage renal disease. *N Engl J Med.* 1982;306:1276–1279.
6. Weir MR. Salt intake and hypertensive renal injury in African-Americans: a therapeutic perspective. *Am J Hypertens.* 1995;8:635–644.
7. Weber M. Interrupting the renin-angiotensin system: the role of angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists in the treatment of hypertension. *Am J Hypertens.* 1999;12:189S–194S.
8. Collins R, MacMahon S. Blood pressure, antihypertensive drug treatment and the risks of stroke and of coronary heart disease. *BMJ.* 1994;14(suppl 1):S39–S46.
9. MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, Abbott R, Godwin J, Dyer A, Stamler J. Blood pressure, stroke, and coronary heart disease, part 1: prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet.* 1990;335:765–774.
10. He J, Whelton P. Elevated systolic blood pressure as a risk factor for cardiovascular and renal disease. *J Hypertens.* 1999;17(suppl):S7–S13.
11. Grimm R Jr, Flack J, Byington R, Bond G, Brugger S. A comparison of antihypertensive drug effects on the progression of extracranial carotid atherosclerosis: the Multicenter Isradipine Diuretic Atherosclerotic Study (MIDAS). *Drugs.* 1990;40:38–43.
12. Samuelsson O, Wilhelmsen L, Svardsudd K, Pennert K, Wedel H, Berglund G. Mortality and morbidity in relation to systolic blood pressure in two populations with different management of hypertension: the study of men born in 1913 and the multifactorial primary prevention trial. *J Hypertens.* 1987;5:57–66.
13. Black H. Optimal blood pressure: how low should we go? *Am J Hypertens.* 1999;12:113S–120S.
14. Dawber T. *The Framingham Study: Epidemiology of Atherosclerotic Disease.* Cambridge, Mass: Harvard University Press; 1980.
15. Neutel J, Smith D, Weber M. Is high blood pressure a late manifestation of the hypertension syndrome? *Am J Hypertens.* 1999;12:215S–223S.
16. Seedat Y. Improvement in treatment of hypertension has not reduced incidence of end-stage renal disease. *J Hum Hypertens.* 1999;13:747–751.
17. Trollet MR, Rudd MA, Loscalzo J. Oxidative stress and renal dysfunction in salt-sensitive hypertension. *Kidney Blood Press Res.* 2001;24:116–123.
18. Trollet MR, Forde P, Rudd MA, Loscalzo J. Effect of antioxidants on salt-induced hypertension and renal dysfunction. *Hypertension.* 2000;36:685c.
19. Brewster UC, Perazella MA. The renin-angiotensin-aldosterone system and the kidney: effects on kidney disease. *Am J Med.* 2004;116:263–272.
20. Granger CB, McMurray JJ, Yusuf S, Held P, Michelson EL, Olofsson B, Ostergren J, Pfeffer MA, Swedberg K. Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function intolerant to angiotensin-converting-enzyme inhibitors: the CHARM-Alternative trial. *Lancet.* 2003;362:772–776.
21. Verma S, Strauss M. Angiotensin receptor blockers and myocardial infarction. *BMJ.* 2004;329:1248–1249.
22. Cheung BM, Cheung GT, Lauder IJ, Lau CP, Kumana CR. Meta-analysis of large outcome trials of angiotensin receptor blockers in hypertension. *J Hum Hypertens.* 2006;20:37–43.
23. Julius S, Kjeldsen SE, Weber M, Brunner HR, Ekman S, Hansson L, Hua T, Laragh J, McInnes GT, Mitchell L, Plat F, Schork A, Smith B, Zanchetti A. Outcomes in hypertensive patients at high cardiovascular risk treated with regimens based on valsartan or amlodipine: the VALUE randomised trial. *Lancet.* 2004;363:2022–2031.
24. Schmidt BM, Schmieder RE. Aldosterone-induced cardiac damage: focus on blood pressure independent effects. *Am J Hypertens.* 2003;16:80–86.
25. Carey R, Wang Z-Q, Siragy H. Role of the angiotensin type 2 receptor in the regulation of blood pressure and renal function. *Hypertension.* 2000;35:155–163.
26. Horiuchi M, Akishita M, Dzau V. Recent progress in angiotensin II type 2 receptor research in the cardiovascular system. *Hypertension.* 1999;33:613–621.
27. Kakuchi J, Ichiki T, Kiyama S, Hogan B, Fogo A, Inagami T, Ichikawa I. Developmental expression of renal angiotensin II receptor genes in the mouse. *Kidney Int.* 1995;47:140–147.
28. Wang Z-Q, Moore A, Ozono R, Siragy H, Carey RM. Immunolocalization of the subtype-2 angiotensin II (AT₂) receptor protein in rat heart. *Hypertension.* 1998;32:78–83.
29. Senbonmatsu T, Ichihara S, Price E Jr, Gaffney FA, Inagami T. Evidence for angiotensin II type 2 receptor-mediated cardiac myocyte enlargement during in vivo pressure overload. *J Clin Invest.* 2000;106:R25–R29.
30. Ito S, Arima S, Ren Y, Juncos LA, Carretero OA. Endothelium-derived relaxing factor/nitric oxide modulates angiotensin II action in the isolated microperfused rabbit afferent but not efferent arteriole. *J Clin Invest.* 1993;91:2012–2019.
31. Siragy H, Carey R. The subtype-2 (AT₂) angiotensin receptor regulates renal guanosine cyclic 3', 5' monophosphate and AT₁ receptor-mediated prostaglandin E₂ production in conscious rats. *J Clin Invest.* 1996;97:1978–1982.
32. Lim PO, Farquharson CA, Shiels P, Jung RT, Struthers AD, MacDonald TM. Adverse cardiac effects of salt with fludrocortisone in hypertension. *Hypertension.* 2001;37:856–861.
33. Rudd MA, Daumerie G, Maitland K, Bridges L, Yancey S, Cloutier T, Toolan G, Davis W, Huang P, Loscalzo J. Increased glomerular damage during high salt diet in eNOS deficient mice. *Circulation.* 2003;108:IV-269.
34. Ahn J, Varagic J, Slama M, Susic D, Frohlich ED. Cardiac structural and functional responses to salt loading in SHR. *Am J Physiol.* 2004;287:H767–H772.

CLINICAL PERSPECTIVE

The results of this study reveal that angiotensin type 1 (AT₁) receptor blockade reduces cardiac hypertrophy and tissue damage in the L-nitroarginine methyl ester (L-NAME) model of hypertension. This effect occurred independently of the antihypertensive action of AT₁ blockade. In contrast, AT₁ receptor inhibition in the Dahl salt-sensitive model did not reduce the tissue injury normally seen in this model despite reductions in blood pressure. However, AT₁ receptor blockade was associated with reduced cardiac hypertrophy but only at the higher dose of receptor antagonist in the L-NAME model. These data invoke several provocative explanations for organ damage associated with hypertension. First, hypertrophy and tissue injury involve countereffects of AT₁ activation, reiterating the complexity of organ damage pathogenesis. Second, hypertension does not necessitate organ damage. The processes involved in hypertension development may occur in parallel with organ damage after a common or separate initial insult. Finally, the etiology of the hypertension determines the effectiveness of antihypertensive treatment. Although the various classes of antihypertensive agents may be equally effective in lowering blood pressure, they may not be equally effective in reducing or preventing organ damage associated with the hypertension. The mechanism(s) of organ damage development will determine which antihypertensive agent would be most efficacious in preventing tissue injury. Therefore, if the objective in treating hypertension is to reduce organ damage, then the etiology of the organ damage rather than simply hypertension itself must be an important determining factor in selecting an antihypertensive agent. In summary, angiotensin receptor blockade appears to protect against organ damage in hypertension that is not salt-dependent and involves reduced NO bioavailability. However, angiotensin II type 1 receptor blockers offer no protection against, and may worsen, end-organ damage in salt-sensitive hypertension.